Are you scared yet? Variations to cue indices elicit differential prey behavioral responses even when gape-limited predators are relatively small

Madison J. Wagner a,b and Paul A. Moore a,b,c

a Laboratory for Sensory Ecology, Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403, USA; b University of Michigan Biological Station, 9133 Biological Road, Pellston, MI 49769, USA; c J.P. Scott Center for Neuroscience, Mind, and Behavior, Bowling Green State University, Bowling Green, OH 43403, USA

Corresponding author: Paul A. Moore (email: pmoore@bgsu.edu)

Abstract

Anti-predator behavior is often evoked based on measurements of risk calculated from sensory cues emanating from predators independent of physical attack. Yet, the exact sensory indices of cues used in risk assessment remain largely unknown. To examine how different predatory cue indices of information are used in risk assessment, we presented prey with various cues from sublethal gape-limited predators. Rusty crayfish (Faxonius rusticus (Girard, 1852)) were exposed to predatory odors from sublethal-sized largemouth bass (Micropterus salmoides (Lacepède, 1802)) to test effects of changing predator abundance, relative size relationships, and total predator length in flow through mesocosms. Foraging, shelter use, and movement behavior were used to measure cue effects. Foraging time depended jointly upon predator abundance and total predator size ($p = 0.030$). Specifically, high predator abundance resulted in decreased foraging efforts as gape ratio increased. Similarly, sheltering time depended on the interaction between predator abundance and gape ratio when predator abundance was highest ($p = 0.020$). Crayfish significantly increased exploration time when gape ratio increased ($p = 0.010$). Thus, this study shows crayfish can use different indices of predatory cues, namely total predator abundance and relative size ratios, in risk assessment but do so in context-specific ways.

Key words: chemosensory landscape, landscape of fear, optimal foraging theory, threat perception, risk assessment, predator–prey ecology, rusty crayfish, Faxonius rusticus, largemouth bass, Micropterus salmoides

Résumé

Un comportement anti-prédateur découle souvent de mesures du risque calculé à partir de signaux sensoriels émanant de prédateurs indépendants de toute attaque physique. Les indices sensoriels exacts des signaux utilisés dans l’évaluation du risque demeurent cependant largement inconnus. Afin d’examiner comment différents indices d’information sur les signaux de prédateur sont utilisés dans l’évaluation du risque, nous avons présenté à des proies différents signaux de prédateurs sublétaux à ouverture buccale restreinte. Des écœuvisses à taches rouges (Faxonius rusticus (Girard, 1852)) ont été exposées à des odeurs d’achigan à grande bouche (Micropterus salmoides (Lacepède, 1802)) de taille sublétale afin de vérifier les effets de variations de l’abondance de prédateurs, des relations des tailles relatives et de la longueur totale des prédateurs dans des mécosomes à écoulement continu. La quête de nourriture, la mise à l’abri et le comportement de déplacement ont été utilisés pour mesurer les effets de signaux. Le temps d’alimentation dépend à la fois de l’abondance de prédateurs et de la taille totale des prédateurs ($p = 0.030$). Plus précisément, une forte abondance de prédateurs se traduit par une diminution des efforts de quête de nourriture à mesure que le rapport d’ouverture buccale augmente. De même, le temps passé à l’abri dépend de l’interaction entre l’abondance de prédateurs et le rapport d’ouverture buccale quand l’abondance de prédateurs est maximum ($p = 0.020$). Les écœuvisses prolongent significativement le temps d’exploration quand les rapports d’ouverture buccale sont plus grands ($p = 0.010$). L’étude démontre donc que les écœuvisses peuvent utiliser différents indices de signaux de prédateur, notamment l’abondance totale de prédateurs et les rapports des tailles relatives, dans l’évaluation du risque, mais que cette utilisation dépend du contexte. [Traduit par la Rédaction]
Introduction

Over the last two decades, the landscape of fear concept has dominated thinking within predator–prey studies (Preisser et al. 2005; Gaynor et al. 2019). This concept centers on the idea that a prey’s behaviors are primarily driven by external stimuli or cues that emanate from predators (Lima and Dill 1990). These cues evoke a form of fear or aversion behavior in prey, and that behavior ultimately results in ecological consequences across trophic levels. Prey may use information contained within sensory stimuli in the landscape of fear by increasing vigilance, altering movement patterns, and modifying foraging choices (Ripple and Beschta 2004; Tolon et al. 2009; Laundré et al. 2010). Also, the decisions of consumer species to alter foraging in light of predation can change plant biomass, species richness, and nutrient cycling (Schmitz et al. 1997; Fukami et al. 2006; Greig and McIntosh 2006). In particular, prey distribution within habitats is largely based upon the perceived level of threat within a given area and the movement away from high threat or risk locales. Prey animals extract meaningful information from these predatory cues and use that information in the decision-making process (Gallagher et al. 2017). Underlying these ecological interactions is the interaction between a prey’s sensory abilities and the dynamic spatial and temporal distribution of predatory cues from which the prey extract information. This spatial and temporal distribution of predator cues is a smaller part of the entire sensory landscape of prey organisms.

A sensory landscape describes the spatial and temporal distribution of stimuli in a given environment, which can be affected by both biotic and abiotic factors (Wilson and Weissburg 2013; Lunt and Smee 2015; Clark and Moore 2018). The sensory landscape is composed of information contained in the intensity, spatial and temporal distribution, and elemental composition of the cues themselves, such as the combination of aromas, colors, and different sounds of sensory stimuli (Fine et al. 2010). While a number of studies have been conducted illustrating the numerous ways animals use the sensory landscape to make a host of decisions (Lass and Spaak 2003; Boeing et al. 2006; Brown and Chivers 2006), work on the landscape of fear has focused on correlating predator presence and movement with prey behavior without detailing how that predator presence creates dynamic sensory landscapes. If we imagine a single large predator, say an alligator gar, swimming through a water system, the spatial and temporal distribution of visual, auditory, chemical, and even vibratory cues will be significantly different. For example, the downstream plume of the gar’s odor will likely carry further, but slower, than any visual cue which will be equally distributed in 360 degrees around the gar (Fischer et al. 2017). Prey relying on vision may have a different landscape of fear as opposed to those that rely more heavily on olfaction. Often, organisms will experience multimodal sources of sensory stimuli at once (e.g., vision and audition) as well as multiple elements of cues in a single mode (e.g., the intensity of the roar as well as the duration). In reality, the landscape of fear is not just the spatial distribution of stimuli, but the interaction of that distribution with a prey’s dominant sensory modality and sensitivity to the stimuli, among other sensory aspects.

Various sensory modalities, either in conjunction with one another or singularly, are able to provide prey with important information about the surrounding environment. These modalities can include vision, audition, olfaction, taste, and pressure (Bouwma and Hazlett 2001; Smolka et al. 2011). In addition to these singular modes, organisms use sensory information gathered from multiple modes for a more accurate description of the surrounding environment (Lukas et al. 2021). Within the landscape of fear concept, these sensory mechanisms have evolved to detect the degree of threat or risk associated with the presence of predators. Different aspects of that threat can be inferred, such as predator diet and the satiation level of the predator, from different sensory cues (Smith and Belk 2001). When organisms are presented with numerous and sometimes conflicting environmental stimuli along with sensory noise, relevant information on the level of threat associated with predatory cues must be extracted to optimize an organism’s chances to avoid predation and be successful in foraging attempts (Hazlett, 1999; Bytheway et al. 2013). Colorado potato beetle (Leptinotarsa decemlineata (Say, 1824)) larvae, when presented with chemical, tactile, and visual predatory information, rely most heavily on predator odor recognition to accurately identify the spined soldier bug (Podisus maculiventris (Say, 1832)), a natural predator (Herrmann and Thaler 2014). Atlantic salmon (Salmo salar Linnaeus, 1758) displayed the highest level of anti-predator behaviors when presented with both physical and chemical predatory cues as opposed to just one type of cue (Blanchet et al. 2007). The mechanisms by which information is perceived and acquired is an important factor for prey survival. Thus, threat perception is the currency with which animals make decisions on how to allocate time and space as well as how to compare risk versus reward.

However, predatory cues are made up of a wealth of components that prey animals must decipher to make advantageous decisions in threatening situations (Schoepfner and Relyea 2005). The components of the cues or signals are indices of information about the origin of the sensory stimuli (Maynard Smith and Harper 2003). Some of the components contained within these predatory cues may include the concentration/intensity of the cues (Holopainen et al. 1997; Zhoa and Chivers 2005), duration of the cues (Peacock 2006), relative concentration of different cue components, the number of cues present (Weiss et al. 2012), and distance from the cue source (Wisenden et al. 2010). In these examples, the signal intensity may be an index of the size of the signal source (Maynard Smith and Harper 2003). These components all signal information on the levels of potential threat to prey species. Yet not all of these components carry equally valued information on predation threats. Identification of the preda-
tor and its location may not provide the prey with all of the salient information to make effective behavioral decisions (Herberholz and Marquart 2012). A satiated predator close to the prey may be less of a threat than a hungry predator farther away (Häberli et al. 2010). In gape limited predators, the relative size of the predator to prey is an important piece of information about the threat (Shine and Sun 2003). Even if the predator is smaller than the prey, a greater abundance of predators may signal higher threat levels. Striped field mice (Apodemus agrarius (Pallas, 1771)) examined the risk of being bitten by red wood ants (Formica polyctena Foerster, 1850 or Formica aquilonia Yarrow, 1955), as well as the reward (the consumption of the ants) by choosing to hunt in tunnels that contained relatively fewer ants (Panteleeva et al. 2013). Similarly, giraffes have been known to alter resource use when lions are nearby even though there is a large size difference between these animals (Strauss and Packer 2012). The ability to determine threat levels hinges on an animal’s ability to pick out which pieces of environmental information are relevant at a specific point in time (Stevens 2013).

While many organisms use multimodality to determine threat, in many aquatic environments, predator–prey interactions are generally mediated by chemical cues (Brönmark and Hansson 2000; Wisenden 2000; Ferland-Raymond et al. 2010). Chemical cues may enter the environment in several ways including from the predator’s skin (Glover et al. 2013), gills (Evans et al. 2005), and excrement (Brown et al. 1995). The chemical cues that naturally emanate from a predator’s body alert prey species to the presence of the predator and provide relevant information about the predator, including relative size ratios (Eklöv 2000). For instance, cues emanating from multiple predators may be perceived as more threatening than a lone predator independent of the size of the predators (Rahel and Stein 1988). However, when taking into account the relative size ratios of predator and prey, if predators are smaller relative to prey, predator abundance may be a more unimportant component, and the predatory information may be seen as less threatening (Choh et al. 2012). Thus, prey may use components of chemical cues in conjunction with another sensory cue as measures of indices of the source to make decisions or prey may pull apart cue components and, situationally, respond to only one specific piece at a time.

However, some organisms rely most heavily on one sensory modality to make relevant decisions. For instance, in aquatic systems, crayfish primarily use chemical signals and cues to navigate habitats (Callaghan et al. 2012; Driscoll et al. 2020). Crayfish have evolved a highly sensitive chemosensory system that allows for the detection of predators in dark waters where visual cues may be unhelpful and less accurate and have been established as ecosystem engineers as well as keystone species (Jones et al. 1994; Statzner et al. 2000; Creed and Reed 2004; Usio and Townsend 2004; Wright and Jones 2006). Previous work has shown that crayfish are able to infer the diet, and hence threat, of predators based off chemical stimuli only (Beattie and Moore 2018). Crayfish are also able to distinguish relative size ratios between themselves and predators solely based on the chemical cues available in the environment (Wood and Moore 2019, 2020). Thus, this lends evidence that in situations where crayfish have no visual stimuli about a potential threat, they are still able to gather information about predation threats through the chemical cues contained in water.

Though previous experiments have assessed predator–prey interactions based on effects that predators have on prey species’ behavioral responses, changes in prey behavior due to detailed changes in cue elements beyond diet, such as the relative size relationships between predator and prey as well as predator abundance, have remained overlooked. For example, is it possible for prey to determine the number of distinct predators, and different degrees of threat, from chemical cues? In the crayfish-bass predator prey system, more bass should increase the threat response from crayfish, but can crayfish extract an estimate of the number of bass from chemical cues. If the amount of predatory cue is related to the total surface area of a predator and this remains constant across situations, can crayfish distinguish between a high threat situation (one large bass), a medium threat situation (three smaller bass), and a nonthreat situation (many juvenile bass) from chemical cues alone? This project sought to uncover how altering three different cue indices independently induced behavioral responses in prey. The first of the three cue indices is the relative size of the predator to the prey, as bass are a gape-limited predator. The second and third indices are the number of predators as well as the total length of predators present. These two components modulate the total surface area of predator exposure to water. As the major source of predatory cues is skin, altering the total surface area of predators in an experiment should, by proxy, alter the total concentration of predatory cues in the water (Glover et al. 2013). Specifically, we were interested in testing whether prey would respond to three relatively small predators with the same magnitude as when exposed to one larger predator.

Largemouth bass (Micropterus salmoides (Lacepède, 1802)) were selected as predators because bass are gape-limited predators and are found throughout much of North America in cooccurrence with crayfish (Nowlin et al. 2006; Christensen and Moore 2009). As discussed previously, crayfish primarily use chemosensation to navigate through habitats. Rusty crayfish (Faxonius rusticus (Girard, 1852)) are also a natural food source of largemouth bass. Thus, crayfish were selected as the prey species in this experiment. Previous studies have shown that rusty crayfish alter foraging behaviors and habitat usage patterns when threatened by predation from one or more predators (Blake and Hart 1993; Shave et al. 1994). Even in situations where predators are relatively small, prey have been known to increase vigilance and participate less in risky behaviors, such as foraging (Hill and Weissburg 2013). Thus, we hypothesized that prey animals exposed to cues from relatively small adult predators would decrease foraging efforts when gape ratio, predator abundance, and total predator length were increased, as independently from one another as possible, because the total accumulation and presence of predatory cues would still trigger fear responses. We also expected that prey would choose to spend more time actively sheltering when exposed to cues.
Materials and methods

Experimental design

To test these ideas, we constructed an experiment where we measured the behavioral responses of crayfish to alterations in chemical cues emanating from predators. We altered these sensory stimuli by changing three different factors that would contribute to the magnitude and intensity of chemical cues created in our mesocosms. The three different components were the number of predators present, the summed total length of the predators present, and the average gape ratio of the predators present. The number of predators serves as a proxy manipulation of the overall surface area of predator skin available to release predatory odors (Wisen- den 2015; Wood and Moore 2020). Number of predators ranged from zero (controls) to a maximum of three. In a similar way, changing the summed total length of the predators in a trial also alters the overall surface area for the release of chemical cues. Summed total lengths of predators ranged from 44.9 to 80.6 cm. These two variables (number of predators and summed total length of predators) alter skin surface area in two different ways, as a singular large predator’s surface area could be equivalent to three small predators’ combined surface area. Finally, since these predators are gape limited, we measured the average gape ratio of predators present in a trial. Bass were chosen in a pseudorandom way, as we wanted to have a range of gape ratios present in this study. Gape ratios ranged from 0.311 to 0.753 and were calculated by dividing the gape of the bass by the carapace width of the crayfish and taking the average of this measure if multiple bass predators were present (Wood and Moore 2020). Historically, gape ratios of less than 0.9 for these predators indicated that the bass cannot consume or swallow the crayfish (Hill et al. 2004). The response to these altered threats was based upon changes in crayfish responses within a mesocosm. Behavioral measures included foraging, shelter use, and general movement variables. Within each mesocosm, a single crayfish was used in the behavioral assay regardless of the number of bass present in the predatory section of the mesocosm. Given the small numbers of bass available due to the COVID-19 pandemic effects on fish farms, bass were reused but crayfish were not. Bass were placed in randomized mesocosms each time, and groupings of bass varied across trials. Bass abundance treatment was also randomized throughout the experimental period. Because of the limited number of bass in this study, it was not possible to obtain equivalent replicates of bass abundance across trials. These differences were taken into account when running statistical analyses. A total of 83 trials were run: 10 with no bass (controls), 30 trials were run with a single bass, 19 trials were run with two bass, and 24 trials were run with three bass.

Collection and housing of animals

Two-hundred form II (nonreproductive) male rusty crayfish (carapace width = 1.26 ± 0.026 cm (mean ± SE)) were captured using minnow traps baited with sardines (Beach Cliff® sardines in soybean oil) from Carp Lake River in Emmet County, Michigan, USA (45.7497°N, 84.7738°W). Crayfish served as sources of predatory fish odors. The fish were purchased from Imlay City Fish Farm Inc. (Imlay City, MI, USA). Fish were fed a diet a commercial fish food (Sportsman’s Choice® Trophy Fish Feed). Upon completion of the trials, bass were euthanized following the approved Institutional Animal Care and Use Committee (IACUC) protocol. Fish were stored in two separate flow-through cattle troughs, using water from the East Branch of the Maple River where largemouth bass naturally occur. The troughs (200 cm × 60 cm × 60 cm (length × width × depth)) used PVC pipes to deliver unfiltered river water to the systems as well as standpipes that kept the water depth at approximately 60 cm. Each trough also contained PVC pipes (7.6 cm diameter) that fish used as refuges. All fish and crayfish were kept outdoors under the natural temperature and daylight:darkness regime. Water temperatures fluctuated from 19 to 22 °C throughout the experimental period. The natural daylight:darkness regime in northern Michigan consisted of 15.5 h of light and 8.5 h of darkness. Because treatments were randomized throughout the summer, any discrepancies due to water temperature or light were evenly spread across all treatments.

Ethical approval

Largemouth bass were maintained and handled following established animal care and use procedures. The use of vertebrate animals was approved by the IACUC at University of Michigan (protocol PRO00008892) and by IACUC at Bowling Green State University (protocol 1411240-6).

Plant collection and storage

Samples of common muskgass (genus Chara Linnaeus, 1763) were collected from North Fishtail Bay of Douglas Lake, in Cheboygan County, Michigan, USA (45.5618°N, 84.6762°W). A macrophyte sampling rake, made by tying the heads of two rakes together so that the tines point outwards and attaching them to rope, was cast into mats...
of submerged vegetation to collect the aquatic plants. The collected macrophytes were held in one half head tank (59.1 cm × 59.1 cm × 43.5 cm (length × width × height)) that acted as a storage stream and was filled with water from the East Branch of the Maple River. The half head tank had an overflow hole drilled into the side that allowed for water to constantly flow through. The macrophyte storage stream was placed in open sunlight to mimic a natural environment. A surplus of macrophyte samples was maintained, and macrophytes were cycled through the storage tank every 2 weeks from 5 June 2021 until trials ended on 22 July 2021.

Experimental mesocosms

Cinderblocks were used to frame eight flow-through stream mesocosms (223.52 cm × 121.92 cm × 40.64 cm (length × width × depth)) which were lined with 6-mil polyethylene sheeting. A pair of 208 L plastic drums served as constant head tanks for the eight mesocosms and were filled with unfiltered water from the East Branch of the Maple River. Water entered into the drums via 7.6 cm PVC pipes that used nylon stockings to filter out macroinvertebrates. Each plastic drum fed four mesocosms with water using two garden hoses per mesocosm; hoses had diameters of 1.9 cm (flow rate = 0.1 ± 0.05 L/s (mean ± SE)). Each mesocosm (Fig. 1) was comprised of a predator arena and a prey arena. Predator arenas were covered with egg crating to prevent bass escape and were always upstream of the prey arenas. The predator and prey arenas of each mesocosm were measured at 111.76 cm × 60.96 cm × 20.32 cm (length × width × depth). The prey sections were lined with sand substrate (depth = 5.1 cm) which accumulated fine detrital material and provided a dark background against which the crayfish were easily observed in video recordings. This same construction technique has been used successfully in previous experiments (Beattie and Moore 2018; Wood et al. 2018; Wood and Moore 2019). Water flowed into the upstream predator section of each mesocosm before overflowing through a screened opening (28 cm × 12 cm opening with 1 mm × 1 mm screening) in a partial wall into the downstream prey section. The water overflowing through the screened opening did not exceed 5 mm in depth, which is an inadequate depth for crayfish to see bass in the predator section of the arena (Wood and Moore 2020). The water exited from the downstream end of the mesocosm through another screened opening. A single PVC half-pipe shelter (10 cm × 8.5 cm × 4 cm (length × width × height)) with one opening was placed near the down current end of the prey section.

A wooden frame held an infrared DVR camera (Zosi ZR08ZN10) 1.3 m above the water surface of each mesocosm to record the crayfish’s nocturnal behaviors. Cameras had a frame rate of 30 frames/s, which is high enough to capture crayfish movement (Moore et al. 2021). One low-intensity red light bulb (Great Value brand: Model A19045 LED Lamp, 9 W, 145 mA, 120 V, 60 Hz, RED) was used to illuminate each mesocosm from above. Crayfish behavior is not impacted by the presence of red light due to crayfish insensitivity to red light wavelengths (Cronin and Goldsmith 1982; Bruski and Dunham 1987). An awning made from a black utility tarp (6.1 m × 12.2 m) covered all mesocosms to prevent weather and water damage to the electrical equipment. The awning also eliminated glare from moonlight and starlight from the recordings. Sunlight was able to enter the system through 1.5 m openings located on the end of all downstream prey arenas, meaning sunlight exposure and water temperature in mesocosms remained similar throughout the experimental period.

Experimental protocol

Each trial was run for 23 h beginning on 7 June 2021 and concluding on 22 July 2021. Trial cycles began at 08:30 with the selection and measurement of bass from the flow-through cattle streams. Bass were removed from the holding tanks and measured on a fish board to find total length to the nearest 0.1 mm. Bass were then placed into the predator arenas of the mesocosms. Bass abundance was randomized as was bass placement in mesocosms. Next, a single crayfish was selected for each stream, meaning that all experimental mesocosms had a singular crayfish during the time of the trial. Crayfish carapace width (1.26 ± 0.026 cm (mean ± SE)) was measured to the nearest 0.5 mm using calipers before crayfish were added to the prey arena. Crayfish were marked with a triangular white patch on their carapace before each trial using a nontoxic correction pen (BIC® Wite-Out® 2 in 1 Correction Fluid) to improve visibility for tracking in video recordings. The behavior of crayfish is not altered by the presence of Wite-Out application (Fero and Moore 2008; Martin and Moore 2008; Jurcak and Moore 2018). Last, stems of Chara weighing approximately 5 g (5.00 ± 0.005 g (mean ± SE))

Fig. 1. Flow-through stream mesocosm. Each mesocosm contained macrophytes were held in one half head tank from a garden hose into the upstream predator arena (orange), where largemouth bass (Micropterus salmoides) were kept to serve as odor donors. Water and odors then entered into the downstream prey arena through a screen where a single rusty crayfish (Faxonius rusticus) was kept, as well as the macrophyte Chara to serve as a foraging assay. Each prey arena had three zones (the foraging zone (plain), the neutral zone (cross-hatched), and the shelter zone (diagonal lines)) allowing for quantification of crayfish behavioral responses. Water then exited the mesocosm through a screen in the prey arena. [Color online.]
in total were selected for each trial. Excess surface water was removed from each plant sample by spinning selected macrophytes in a salad spinner (Farberware Basics, Item No. 5158683) for 20 rotations before weighing to the nearest 0.001 g. The macrophyte stems were then attached to glass rods (255 mm × 6 mm (length × outer diameter)) with 26-gauge green painted floral wire. The loaded rods were placed into a hardware cloth bracket (24 cm × 19 cm (length × width)) which held the samples in position during the feeding trial. Because macrophytes were wrapped around glass rods before being placed in the hardware cloth brackets, macrophytes would not have offered significantly more habitat complexity as compared to other areas of the mesocosm. This technique has been successful in the past (Wood and Moore 2019, 2020).

Beginning at 2300, an automatic light timer activated the red lights illuminating the mesocosms. At 0000, the cameras above each mesocosm began recording the nocturnal behaviors of the crayfish. The cameras shut down at 0340 when behavioral recordings were complete. This time frame has been shown to be sufficient when observing the nocturnal behaviors of crayfish (Wood and Moore 2019, 2020). Water flow through the mesocosms was slow enough that there was no visible surface distortion in the video recordings. All crayfish were removed from the mesocosms first on the following morning, followed by largemouth bass. Last, Chara samples were removed from each mesocosm and surface dried in the salad spinner again before being weighed a second time to find final mass. Upon completion of trials, the screened openings between the predator and crayfish section and the outflow from the crayfish section were brushed to remove any debris that might inhibit water/odor flow. Because only four trials were run each day, mesocosms were allowed to flush throughout the experiment. This process was used across all trials.

**Crayfish behavior analysis**

Each 3 h 40 min video clip per trial was assessed by a viewer who was blind to treatment (i.e., the viewer could not see into the predator section). The viewer scored the crayfish for its total time spent within the three zones (the foraging zone, sheltering zone, or the clear zone) located in the prey arena. The camera captured images at 30 frames/s and usually crayfish movement is digitized at 1 frame/s (Moore et al. 2021). Because crayfish feeding appendages are located on the underside of the animal, it was not possible to see when the crayfish was actually consuming the macrophytes. Still, crayfish were scored as actively foraging when the mark on their carapace was completely inside the foraging zone. Crayfish were also scored as sheltering when the mark was completely within the sheltering zone. Because we were only interested in how crayfish spent time between the zones, we did not observe any anti-predator behaviors in the form of raised chelae.

Foraging effort was calculated by dividing the total time (s) that crayfish spent in the foraging zone by 13200 s (the total time of the video trial) and multiplying the quotient by 100. This resulting percentage is representative of the total time of the 3 h 40 min that the crayfish spent foraging. Time spent sheltering and time in the clear zone was also calculated this way. The number of transitions the crayfish exhibited between the three zones was also assessed. This helped to show the overall activity of the crayfish in the trial. Thus, the behavioral analysis produced four dependent variables: percent time spent in the clear zone, foraging zone, and shelter zone, as well as number of transitions between zones.

**Macrophyte consumption analysis**

Consumption of the macrophyte Chara was assessed using the following formula:

\[
\frac{\text{initial mass} - \text{final mass}}{\text{initial mass}} \times 100
\]

where the initial mass was determined before trials began, and the final mass was taken on the following day when trials ended. Macrophyte consumption was made into a percentage to account for any possible minor discrepancies in masses throughout the experiment. This process was used across all trials.

**Statistical analysis**

Dependent variables consisted of percent consumption of macrophyte, the percent time spent in each zone (neutral, foraging, sheltering), and the number of transitions between each zone. Data conditioning and treatment followed the steps outlined in Zuur et al. (2009) for mixed effects models. The first step in this process was creating dot charts to examine potential outliers within the dataset. None of the 83 trials had outliers, so all of the raw data were included in further analysis. A collinearity analysis was performed between the independent variables of bass abundance, summed total length of predators, average gape ratio, and carapace width of the crayfish. Carapace width of the crayfish was used to calculate gape ratios. The two variables, bass abundance and summed total length of predators, were highly correlated and, because of this, were never run in the same statistical models. Next, histograms, q–q plots, and Shapiro–Wilks tests of normality were used to examine the underlying distribution of response variables. The percent time in the neutral zone and the number of transitions between the zones were normally distributed. For those variables that were not normal (percent consumption, time spent in the foraging zone and time spent in the shelter zone), “BestNormalized” was run to determine which data transformation was likely to produce the best normalized dataset (Peterson 2021). The consumption of macrophytes was not normally distributed, so a Yeo–Johnson transformation was used on this dataset. The percent time that crayfish used the foraging zone and the time spent in the shelter zone were not normally distributed, so a square root transformation was applied to these data.

Once data conditioning was finished, dependent variables were analyzed using a linear mixed effects model by running the “lmer” function from the “lme4” package in R (Kuznetsova et al. 2017; R Core Team 2019) for all behavioral responses as well as macrophyte consumption. Following appropriate model selection as outlined in Zuur et al. (2009) and recognizing that the bass abundance variable and summed...
total length variable were collinear, a top-down model selection process was used. This was a four-step process where (1) the initial two models contained the full interactions of the independent variables (using bass abundance and not summed total length) as well as the random effects of stream mesocosm (this step involved the construction of two separate models one with bass abundance and one with summed total length given their collinearity), (2) the judgment of the optimal model using the lowest AICs, (3) statistical output of the optimal model by plotting the residuals against the fitted parameters (Zuur et al. 2009). Following model construction, the outputs were extracted using the “summary” and “anova” function from R (Fox and Weisberg 2019). If a significant difference in an interaction term was found, a subsequent regression analysis was used to determine a significant linear relationship between the dependent variable and average gape ratio. This analysis was performed in OriginPro version 2021b (Origin Lab Corporation, Northampton, MA, USA).

Results

Model selection outcomes

The optimal model for all dependent variables used bass abundance instead of the summed total predator length (Table 1). All ΔAIC values between these two models were greater than two so all subsequent analysis disregarded summed total predator length and focused on those mixed models with bass abundance and gape ratio.

Given the limited availability of bass, the range of total lengths of bass was smaller than expected. Because of this, the differences in gape ratio may be driven more by changes in crayfish carapace width than changes in the gape of the bass. To examine this concern, models were run by replacing average gape ratio with the crayfish carapace width and were compared using their AIC estimates. None of the models using crayfish carapace had lower AIC values than the optimal models displayed in Table 1.

Crayfish habitat use

Clear zone

Crayfish showed no significant difference in the use of the clear zone area of the mesocosm across any of the treatments when considering either the average gape ratio, changes in bass abundance, or the interaction between these two variables ($F_{1,7.5;0.05} = 0.0069$ for the interaction, $p = 0.93$; Table 2).

Foraging zone

The time crayfish spent in the foraging zone was significantly influenced by the interaction between the average gape ratio and bass abundance ($F_{1,7.5;0.05} = 7.23$, $p = 0.0008$; Fig. 2; Table 2). A subsequent regression analysis indicated that only the trials using three bass yielded a significant fit ($r^2 = 0.19$, $p = 0.03$). The regression lines for trials with a single bass or two bass were not significant ($p = 0.2$ and $p = 0.17$, respectively). Slopes represent the adjusted value. The slopes for the regression lines are $57.7 \pm 44.9$, $68 \pm 46.9$, and $-64.9 \pm 28.3$ (mean ± SE) for bass abundance 1, 2, and 3, respectively. In particular, crayfish spent significantly less time in the foraging zone as average gape ratio increased, but only when three bass were present in the predator section of the mesocosm.

Shelter use

As with the foraging zone, crayfish use of the shelter was significantly impacted by the interaction between the number of bass in a trial and the average gape ratio of those bass ($F_{1,7.5;0.05} = 7.5$, $p = 0.0076$; Fig. 3; Table 2). Slopes represent the adjusted value. The slopes for the regression lines are $-49.3 \pm 50.2$, $-44.8 \pm 57.5$, and $78.9 \pm 31.2$ (mean ± SE) for bass abundance 1, 2, and 3, respectively. Subsequent linear regressions showed that only the trials with three bass had a significant relationship average gape ratio ($F_{3;0.05} = 0.25$, $p = 0.02$). The regression lines for the trials with a single bass or two bass were not significant ($p = 0.33$ and $p = 0.45$ for one and two bass, respectively). Crayfish increased their use of the shelter zone as average gape ratio increased, but only for the treatment with three bass present.

Transitions

Crayfish transitions between each zone, an indication of movement patterns, was only significantly impacted by average gape ratio regardless of the number of bass present in the mesocosm ($F_{1,7.5;0.05} = 13$, $p < 0.001$; Table 2). There was no significant effect due to bass abundance or the interaction between bass abundance and average gape ratio ($p = 0.4$ and $p = 0.87$, respectively; Table 2). A subsequent regression showed that crayfish significantly increased the number of transitions between mesocosm zones as the average gape ratio increased ($r^2 = 0.3$, $p = 0.01$; Fig. 4).

Foraging

Total consumption

Similar to transitions, the total amount of Chara consumed by crayfish was only significantly impacted by average gape ratio ($F_{1,7.5;0.05} = 29$, $p < 0.0001$; Table 2). Subsequent regression analysis demonstrated this ($r^2 = 0.3$, $p = 0.03$). Crayfish significantly increased their consumption of the macrophyte Chara as average gape ratio decreased regardless of the number of bass in the predator section of the mesocosm (Fig. 5).

Discussion

The results of this study demonstrate that crayfish can use different indices of chemical cues to determine predation risk, but do so in context-specific ways. More specifically, we found that crayfish spent less time foraging and more time in the shelter when exposed to changes in bass gape or higher cue concentration in the water due to larger or more abundant bass. Previous studies have indicated the primary importance of gape ratio for gape-limited predators in relation
Table 1. Summary of AIC scores from the linear mixed effects models for each dependent variable.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Model</th>
<th>AIC</th>
<th>ΔAIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent consumption</td>
<td>Gape ratio and bass abundance (full interactions + random effect)</td>
<td>222</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gape ratio and summed total length (full interactions + random effect)</td>
<td>236</td>
<td>14</td>
</tr>
<tr>
<td>Time in clear zone</td>
<td>Gape ratio and bass abundance (full interactions + random effect)</td>
<td>699</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gape ratio and summed total length (full interactions + random effect)</td>
<td>712</td>
<td>13</td>
</tr>
<tr>
<td>Time in foraging zone</td>
<td>Gape ratio and bass abundance (full interactions + random effect)</td>
<td>357</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gape ratio and summed total length (full interactions + random effect)</td>
<td>371</td>
<td>14</td>
</tr>
<tr>
<td>Time in shelter zone</td>
<td>Gape ratio and bass abundance (full interactions + random effect)</td>
<td>309</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gape ratio and summed total length (full interactions + random effect)</td>
<td>322</td>
<td>13</td>
</tr>
<tr>
<td>Number of transitions</td>
<td>Gape ratio and bass abundance (full interactions + random effect)</td>
<td>673</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gape ratio and summed total length (full interactions + random effect)</td>
<td>686</td>
<td>13</td>
</tr>
</tbody>
</table>

Note: Only the top two models are shown for simplicity. Only models where ΔAIC > 2 were considered optimal and were selected for subsequent statistical analysis.

Table 2. Statistical output of generalized linear mixed models for all dependent variables.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Mixed model</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time in clear zone</td>
<td>Average gape ratio</td>
<td>1.77</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Bass abundance</td>
<td>0.1186</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.0069</td>
<td>0.93</td>
</tr>
<tr>
<td>Time in foraging zone</td>
<td>Average gape ratio</td>
<td>17.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Bass abundance</td>
<td>2.54</td>
<td>0.114</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>7.23</td>
<td>0.0008</td>
</tr>
<tr>
<td>Time in shelter zone</td>
<td>Average gape ratio</td>
<td>6.00</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>Bass abundance</td>
<td>4.83</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>7.5</td>
<td>0.0076</td>
</tr>
<tr>
<td>Transitions</td>
<td>Average gape ratio</td>
<td>13.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Bass abundance</td>
<td>0.72</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>0.03</td>
<td>0.87</td>
</tr>
<tr>
<td>Consumption percent</td>
<td>Average gape ratio</td>
<td>29.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Bass abundance</td>
<td>1.19</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>1.98</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Note: This table shows the F and p values that were generated for each dependent variable from the mixed models. Significant models are highlighted in bold and italic typeface.

Fig. 2. Scatter plot showing the percent time that rusty crayfish (Faxonius rusticus) spent in the foraging zone as a function of the average gape ratio of largemouth bass (Micropterus salmoides) in the trial and total number of bass used for each trial. Red solid circles (one bass, N = 30), blue solid triangles (two bass, N = 19), and green inverse solid triangle (three bass, N = 24) represent the total bass abundance per trial. The red, blue, and green lines and shading represents the best fit line and 95% confidence intervals for all bass abundances. The mixed models indicated a significant interaction effect between average gape ratio and number of bass present (p = 0.0008). A subsequent regression analysis showed that only trials with three bass yielded a significant fit. The control condition of no bass is not shown because there is range of average gape ratio in these trials. [Color online.]
Fig. 3. Scatter plot showing the percent time that rusty crayfish (*Faxonius rusticus*) spent in the shelter zone as a function of the average gape ratio of largemouth bass (*Micropterus salmoides*) in the trial and total number of bass used for each trial. Red solid circles (one bass, \(N = 30\)), blue solid triangles (two bass, \(N = 19\)), and green inverse solid triangle (three bass, \(N = 24\)) represent the total bass abundance per trial. The red, blue, and green lines and shading represents the best fit line and 95% confidence intervals for all bass abundances. The mixed models indicated a significant interaction effect between average gape ratio and number of bass present \((p < 0.001)\). A subsequent regression analysis showed that only trials with three bass yielded a significant fit. The control condition of no bass is not shown because there is range of average gape ratio in these trials. [Color online.]

Moore 2020), foraging and movement were significantly influenced by an increase in the gape ratio (or threat). Movement, as measured by transitions between sections, increased as gape ratio increased (Fig. 4), while foraging decreased as the gape ratio increased (Fig. 5). Because the bass were below the gape ratio where attacks on crayfish are successful (approximately 0.9 or higher), we expected that crayfish would have little to no behavioral response to these sublethal threats (Hambright 1991; Hill et al. 2004). However, as the results show, crayfish are responding to more than one source of chemosensory information. In line with previous studies, crayfish respond to increasing bass gape ratio by decreasing foraging time and choosing to shelter more (Wood and Moore 2020). The present study highlights that crayfish shelter more often when bass gape ratio and bass abundance interact, but significance is only seen for the highest bass abundance treatment (Fig. 3). This shows that, although bass abundance is an important factor in crayfish decision-making, bass gape ratio was more likely to influence behavioral changes by causing crayfish to forage less, shelter more often, and increase movement in the system. The change seen in crayfish’s willingness to participate in foraging activities, shelter use, and movement indicates that both predator abundance and relative size comparisons are important factors to this tri-trophic system.

Fig. 4. Scatterplot showing the total number of transitions in each trial as a function of the average gape ratio of each trial. Each green circle represents a single trial \((N = 73)\). The green line and shading represent the best fit line and the 95% confidence interval. The mixed model showed that there were significant increases in the number of rusty crayfish (*Faxonius rusticus*) transitions between zones with increasing average gape ratio of largemouth bass (*Micropterus salmoides*) \((p < 0.001)\). [Color online.]

Fig. 5. Scatterplot showing the total percentage of *Chara* consumed per trial as a function of the average gape ratio per trial. Each green square represents a single trial \((N = 73)\). The green line and shading represent the best fit line and the 95% confidence interval. The mixed model indicated *Chara* consumption significantly decreased as average gape ratio increased \((p = 0.015)\). [Color online.]
The information animals extract from environmental cues is critical in determining risk versus reward (Strohbe et al. 2011). Certainly, the location and value of rewards, such as food and mates, is important within decision making theory, but predator abundance, location, and threat are also important to extract (Corcoran et al. 2013). The measurement of threat, as detected from sensory cues, is not a singular measure and can be considered to contain several dimensions such as spatial location, abundance, hunger level, and size of the predator (Kats and Dill 1997). Because threat represents the potential for danger (Preisser et al. 2005), prey species can investigate threat cues to gain important information about various aspects of the predatory threat (Derby and Steullet 2001). In this study with crayfish, this information is relative size ratios, predator abundance, and overall intensity of the cue, which can be extracted from the total length of predators (Holopainen et al. 1997). Interestingly in our results, all predators were below a size criteria where they could not consume the crayfish in the trials, yet crayfish did show anti-predatory behavior that was influenced by size of the potential predator(s) (Fig. 4), relative size ratios of the potential predator to prey (Fig. 5), as well as overall predator abundance (Fig. 3). In this case, crayfish appear to be extracting multiple pieces of information about threats based on potentially different elements of the chemical cues (e.g., total concentration, ratio of different chemicals, or dynamic elements of odor plumes) Whether crayfish are extracting threat information solely by gape ratio or in combination of gape ratio and overall intensity of the cue is unknown. The results from this specific study do not allow us to differentiate between these two scenarios. Previous work indicates that crayfish respond to the gape ratio, but again, it is unclear how they obtain this information chemically.

Other organisms have shown similar abilities to extract relevant information from multiple modalities as well as multiple pieces of information from a singular modality (Chivers et al. 2012; Weissburg et al. 2014). For instance, Fischer et al. (2017) found that a species of cichlid (Neolamprologus pulcher (Trewavas and Poll, 1952)) is able to use both visual and chemical cues equally efficiently when identifying predators. York et al. (2016) showed that the lateral line analogue is more important for predator detection in young squid (Doryteuthis pealei (Lesueur, 1821) and Lolliguncula brevis (Blainville, 1823)) than visual cues, but juvenile and adult squid rely on the integration of both the lateral line analogue and vision for successful predator detection and evasion. The ability for animals to use multisensory integration is partially shaped by the ability to successfully transfer predatory information from the environment into relevant working knowledge for an animal, and this information is important even in situations where predators are small relative to prey (Pedersen et al. 2003). As shown in the present study, where actual threat may not match perceived threat, prey animals still display a heightened sense of awareness in response to the predatory information contained their sensory landscape. This vigilant behavior, even in the lack of real threat, could induce changes to prey’s behavior and, ultimately, alter ecological interactions.

The present study highlights the importance of predator-prey interactions even in low-risk settings. These settings, where gape-limited predators are too small to consume prey, still produce nonconsumptive changes in prey behavior, notably in foraging behavior and shelter use, due to multiple sources of information contained in chemical cues (Chivers et al. 2008; Ramamonjisoa and Mori 2019a, 2019b). The process by which prey species forage and change herbivory patterns due to the presence or absence of predators can be viewed through the lens of optimal foraging theory and giving up densities (Brown et al. 1999). In this view, predators mediate habitat appeal by producing an ever-changing gradient of risk that prey animals use to construct a landscape of fear (Lima and Dill 1990). This landscape is spatially and temporally dynamic because of predator movement and inherent heterogeneity in sensory cue transmission (Gaynor et al. 2019). As our results indicated, crayfish foraged less when predators were more abundant even though these gape-limited predators were of a sublethal size ratio. Changes to foraging, notably in a species with a large ecological impact, can alter population dynamics and densities, resource availability, ecosystem geography, and overall habitat health (Menge et al. 1994; Lawton and Jones 1995; Jordán et al. 2006). Haggerty et al. (2018) found that the difference in herbivory patterns of seaweed limpets (Lottia insessa (Hinds, 1842); presently known as Discussa insessa (Hinds, 1842)) as a function of señorita fish (Oxyjulis californica (Günther, 1861)) predation had profound effects on feather boa kelp (Egregia menziesii (Turner) Areschoug), a plant that is responsible for habitat formation, showing that predation by the señorita fish indirectly impacted the ability of a vital plant species to thrive. Similarly, Morris and Letnic (2017) found that the removal of an apex predator, the dingo (Canis dingo Meyer, 1793; presently known as Canis lupus dingo Meyer, 1793), from an Australian landscape impacted kangaroo (red kangaroo, Macropus rufus (Desmarest, 1822), and eastern grey kangaroo, Macropus giganteus Shaw, 1790) presence, resulting in changes to plant biomass and growth, soil nutrient availability, and, ultimately, the patch’s ability to accumulate water and other nutrients over time. Evidently, relationships between predators and prey help shape habitats and ecological interactions, and even predators that are small relative to prey that pose little threat are capable of driving change in an ecosystem due to prey behavioral response. Thus, the ability of prey animals to decipher multiple pieces of information from predatory cues, combine these pieces into one larger working frame, and integrate cue information into specific behavioral responses is a phenomenon important on a small scale, such as prey survivability, and at a larger magnitude when examining ecosystem functioning.

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Data availability
Data will be made public and available through the University of Michigan Biological Station data hub, Mfield, upon publication of the article.

Author information
Author ORCIDs
Madison J. Wagner https://orcid.org/0000-0002-9359-3305
Paul A. Moore https://orcid.org/0000-0002-4555-1178

Author contributions
Conceptualization: MJW, PAM
Data curation: PAM
Formal analysis: MJW, PAM
Investigation: MJW
Methodology: MJW, PAM
Supervision: PAM
Validation: MJW
Visualization: PAM
Writing — original draft: MJW
Writing — review & editing: MJW, PAM

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References


